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pH and Chemical Structure

Learning and Lab Skills Objectives

- To put into practice basic safety rules
- To use an analytical balance and a top-loading balance
- To graph data using *Graphical Analysis*
- To measure **pH** using a **pH** meter
- To recognize protonated and unprotonated functional groups
 - carboxylic acid
 - amine
 - carbonate
 - sulfate
 - phosphate
- To tell from a name if a substance is protonated or unprotonated
- To use the Henderson-Hasselbalch equation to:
 - relate relative amounts of protonated and unprotonated forms at a given **pH**
 - to **determine** the pK_a value of an acid
- To relate **pH** and solubility of organic acids
- To identify an unknown acid by titrating a solution containing the acid with NaOH
- To understand buffer capacity

Why Are These Skills Needed?

Understanding **pH** and its effect on the structure, solubility, color, and distribution behavior of chemical substances is perhaps the most important single concept in introductory chemistry. This experiment is designed to make you think about these ideas in a systematic way.

What Will You Do?

The experiment has four parts. In the first experiment you will be given an unknown acid whose concentration is 0.1M. By titrating the solution with 0.1M NaOH you will **determine** if the acid is monoprotated (HA), diprotated (H_2A) or triprotated (H_3A).

In the second experiment, you will distinguish whether or not an unknown solution is saturated sodium benzoate or benzoic acid. Again understanding the properties of protonated and unprotonated forms of

an acid will help you identify your unknown.

In the third experiment, you will **determine** the properties of a buffer solution.

In the fourth experiment, you will be given a similar unknown solution. By titrating the solution with 0.1M NaOH you should be able to estimate the pKa of the acid and **determine** the concentration of the acid. You will also see that at the endpoint, the slope of the graph of **pH** versus volume of NaOH will be a maximum. In all four parts, you will find important clues to interpret your results by reading carefully the background section which follows.

Background:

"Most metabolic reactions are exquisitely sensitive to the hydrogen-ion concentration of the fluid in which they occur. This sensitivity is due primarily to the marked influence on enzyme function exerted by the hydrogen ion. Accordingly, the hydrogen-ion concentration of the extra cellular fluid is one of the most critical and closely regulated chemical quantities in the entire body." (Human Physiology, Vander, Sherman, and Luciano, McGraw Hill, 1975, p 349).

"Exquisitely sensitive" what a wonderful way to describe the centrality of acid/base behavior to the proper functioning of all living systems. Note that we connect hydrogen ion concentration to the concept of acid/base, and to **pH** in a seamless way. It is that total familiarity of these ideas that should be your goal for this module.

Why do chemists (and nearly everyone else) need to know about acids, bases, and buffers?

Here are some interesting facts about acids/bases, **pH** and hydrogen concentration:

- The normal human stomach secretes about 2 liters of hydrochloric acid solution a day.
- The concentration of hydrogen ions in the lumen of the stomach can be as high as 150 mM, nearly 4 million times more concentrated than the concentration of hydrogen ions in blood.
- The movement of hydrogen ions from the inside of the mitochondria to the inter membrane space is the major driving force for the production of all the usable energy in aerobic cells.
- The hydrogen ion concentration in tissue capillaries is higher than in arterial blood. This decreases the ability of hemoglobin to carry oxygen and additional oxygen is released. Conversely, the hydrogen ion concentration in lung capillaries is lower than venous blood so that hemoglobin picks up more oxygen than expected by just the amount of available oxygen.
- The major source of hydrogen ion in the body comes from the reaction of metabolically produced CO₂ with water, catalyzed by the enzyme carbonic anhydrase:
- A high protein diet can cause the release of large quantities of sulfuric acid. The average American diet results in the liberation of 40-80 mmoles of hydrogen ion each day. (Vander, p 353).
- Disease can markedly affect the level of hydrogen ion. For example, an increased hydrogen ion concentration caused by the accumulation of the ketone bodies, acetoacetate and 3-hydroxybutyrate, accompanies diabetes.
- The structures and therefore the function of most of the building-block molecules in biochemistry (amino acids, fatty acids, and nucleic acids, are significantly affected by the concentration of hydrogen ion. Similarly, the inorganic ions in living systems, principally phosphate and bicarbonate, and most drugs from Aarane to Zylprim are also affected by the

concentration of hydrogen ion.

- Blood **pH** (the -log of the hydrogen concentration) is between 7.36 and 7.44. When the **pH** of an individual's blood drops below 7.36, the individual has acidosis. If the **pH** of the blood becomes greater than 7.44, the individual has alkalosis. A blood **pH** compatible with human life is 6.8 to 7.8
- The **pH** of the following biological fluids is: urine <6, gastric juice <1-2, intestinal juice <6.5-7.6, bile from gall bladder <5-6, pancreatic juice <7.6-8.2.

All living systems must regulate the concentration of hydrogen ion to function properly. This is called *homeostasis*. Hydrogen ion homeostasis is regulated by:

- dilution
- buffering action of all three of the following :
 - a) the $\text{H}_2\text{CO}_3/\text{HCO}_3^{-1}$ system
 - b) proteins, specifically hemoglobin in blood
 - $\text{H}_2\text{PO}_4^{-1}/\text{HPO}_4^{-2}$ system
- c) respiration of CO_2 through the lungs
- kidney control of the amount of ammonium ion and weak organic acids

That means that everyone who studies living systems: nurses, biologists, chemists, pharmacologists, athletes, athletic trainers, physicians, gardeners, must have a thorough understanding of acids, bases, and **pH**.

The relation between **pH** and hydrogen concentration, $[\text{H}^+]$

The term **pH** is derived from the French, *l'puissance d'hydrogen*, literally the power of hydrogen ion. **pH** is something that is measured, most commonly with a **pH** meter. There is an inverse relation between **pH** and concentration of H^+ .

high **pH** means low concentration of H^+

low **pH** means high concentration of H^+

- If we add H^+ to a system, we lower the **pH**. We add H^+ when we add acids.
- If we remove H^+ from a system, we increase the **pH**. We remove H^+ when we add bases.

The actual relation between **pH** and H^+ is:

pH = $-\log a_{\text{H}^+}$ which is approximately $\log [\text{H}^+]$

If the **pH** is 7 then the $[\text{H}^+]$ is $1 \times 10^{-7}\text{M}$

If the **pH** is 1 then the $[\text{H}^+]$ is $1 \times 10^{-1}\text{M}$

If the **pH** is 7.4 then the $[\text{H}^+]$ is $1 \times 10^{-7.4}$ or $3.98 \times 10^{-8}\text{M}$

Thinking about what happens when you add acid to water:

Suppose I add 40 mL of 0.01M HCl to 60 mL water having a **pH** of 7

- The concentration of H^+ in the HCl is 0.01M (**pH** = 2)
- The concentration of H^+ in the water is $1 \times 10^{-7}M$ (**pH** = 7)
 - I added $40\text{mL} \times 0.01\text{mmoles/mL} = 0.4$ mmoles of H^+ from the HCl to $60\text{mL} \times 1 \times 10^{-7}\text{mmole/mL} = 6 \times 10^{-8}$ mmoles of H^+ from the water
 - The total mmoles of $H^+ = 0.4 + 6 \times 10^{-8} = 0.4$ mmoles of H^+ in 100 mL of water
 - The concentration of H^+ is $0.4 \text{ mmoles}/100\text{mL} = 4 \times 10^{-3}M$
And the **pH** is 2.4.

So the **pH** increases a little by adding water but not a lot and the affect is caused by dilution not by adding or removing H^+ .

Thinking about what happens when you add acid to a base:

Adding acid to water simply dilutes the acid. But many substances react with acids. Bases react with acids by removing the H^+ ion and making it water



Suppose I add 40 mL of 0.1M HCl to 50 mL of a base having a concentration of 0.02M

- The millimoles of H^+ added is $40 \text{ mL} \times 0.1\text{mmoles/mL} = 4$ mmoles
- We react this with $50 \text{ mL} \times 0.02 \text{ mmoles/mL}$ of base = 1 mmoles of base

The result is analyzed using a mole table:

	$H^+ + B \longrightarrow HB^+$		
<i>initial</i>	4	1	0
<i>extent</i>	-x	-x	x
<i>at end of reaction</i>	4-x	1-x	x
<i>value of x</i>	4	①	
<i>final mmoles</i>	3	0	1

Note that there is acid remaining (3 mmoles) and that all the base is gone and instead the protonated form of the base is the second compound present.

At the point where the mmoles of H^+ is equal to the millimoles of bases, there is an equivalence (because the two are equal) point. At the equivalence point, there is no H^+ and no base but only protonated base.

Many times, the base is the OH^{-1} ion and the protonated base is water. Ideally then the **pH** of the solution in this case is the **pH** of pure water, 7. This rarely happens however as we shall discuss below.

Thinking about what happens when you add hydroxide ion to an unknown acid.

Chemists often add hydroxide ion in the form of sodium hydroxide or potassium hydroxide to acids.

You will do it in experiment 1. If I measure the volume of hydroxide added to a fixed volume of acid, the procedure is called **titration**. Suppose I had a solution whose hydroxide concentration is such that one drop of it would contain 0.1 mmol of OH^- . Now I measure 5 mL of an acid and I add something to the acid that changes color at the equivalence point, the point where the millimoles of hydroxide added is equal to the millimoles of acid that we start with.

- I add 9 drops of the hydroxide solution without a color change but on the addition of the 10th, the color does change. That means that I added 10 drops \times 0.1 mmol/drop = 1 millimole of base to the acid.
- This result implies that I also have 1 millimole of H^+ in the 5 mL of acid.
- The acid concentration would be 1 mmol/5mL = 0.2M.

What if I knew the concentration of the acid and that it was 0.2M in the acid? What would it mean if I did the experiment with 5 mL of acid and needed:

- 20 mL of hydroxide
- 30 mL of hydroxide
- 40 mL of hydroxide

That would mean that there is more H^+ in the 0.2M acid than in the first case. This can happen. We can acids with a general formula:

1. HA with one H^+ for every acid molecule
2. H_2A with two H^+ for every acid molecule
3. H_3A with three H^+ for every acid molecule
4. H_4A with four H^+ for every acid molecule

The experiment with 5 mL of acid and the hydroxide would give 10 drops for case 1, 20 drops for case 2, 30 drops for case 3 and 40 drops for case 4. The formula tells you why.

What would an analysis that requires a different number of drops, say 13, tell you? Not all drops are equal. Also measuring 5 mL of acid is not uniformly accurate. The formulas are whole number multiples of each other. That's the Law of Definite Proportions. Variations from that whole number are due to error not chemistry.

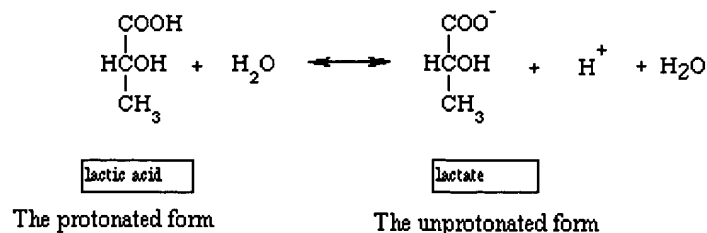
pH affects

- the structure of substances
- the color of substances
- the solubility of substances
- the distribution of a substance between two phases

In the second experiment, you will investigate how the **pH** affects the structure and therefore the solubility of a substance.

The relation between structure and pH.

In water, an acid is a substance that donates an H^+ to water. When it does so, the acid becomes deprotonated (it loses a H^+ , a proton) and the **pH** of the water is lowered (because there are more hydrogen ions. Notice also the charge changes as H^+ is added or removed (not surprising if you think about it). Here is an example, lactic acid. The unprotonated form of lactic acid is lactate.



- If you add acid (H^+) to the system, more lactic acid is formed because the reaction to the left is favored.
- If you remove acid by adding hydroxide ion for example, then the reaction to the right is favored and lactate is formed.

Another way of saying this is:

- lowering the **pH** favors lactic acid formation
- raising the **pH** favors lactate formation

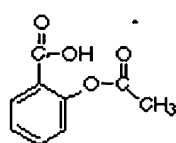
So the structure changes as the **pH** changes.

The relation between solubility and pH.

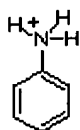
Rule of thumb: Compounds that are charged (either + or -) tend to be more soluble in water than compounds that are uncharged.

pH affects the degree of charge of carboxylic acids and amino groups, common functional groups found in biomolecules and therefore the solubility of these biomolecules.

- Carboxylic acids are uncharged at low **pH** and charged at high **pH**. So carboxylic acids tend to be insoluble at low **pH** and soluble at high **pH**.
- Amines are charged at low **pH** and uncharged at high **pH**. So amines tend to be soluble at low **pH** and insoluble at high **pH**.
- The **pH** value needed to change from high solubility to low solubility is the **pKa**, a property of the acid itself. At **pH** values below the **pKa**, the substance is protonated; at **pH** values above the **pKa** the substance is unprotonated.
- Protonated carboxylic acids are uncharged while protonated amines are positively charged so that under protonation conditions, carboxylic acids are insoluble but amines are soluble.
- Unprotonated carboxylic acids are charged while unprotonated amine are uncharged so that under conditions for unprotonation, carboxylic acids are soluble but amines are insoluble.



aspirin
protonated form
pKa = 3.48

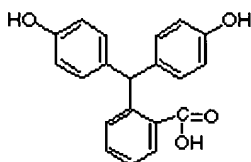


aniline
protonated form
pKa = 4.63

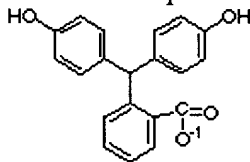
Aspirin is less soluble at pH 2 (less than its pKa of 3.48) while aniline is insoluble at pH 2 because aspirin is protonated and uncharged at that pH while aniline is protonated and charged at that pH.

The relation between pH and color

Many times the protonated form of a colored substance has a different color than the unprotonated form. Compounds called indicators behave that way and complicated natural products that produce color in flowers, fruits, and vegetable can also. Bromocresol green is yellow when protonated and blue when unprotonated (and green when there is a mixture of the two). Phenolphthalein is colorless when protonated



phenolphthalein protonated
colorless



phenolphthalein unprotonated
hot pink

but pink when protonated:

How do we know if something is an acid or a base?

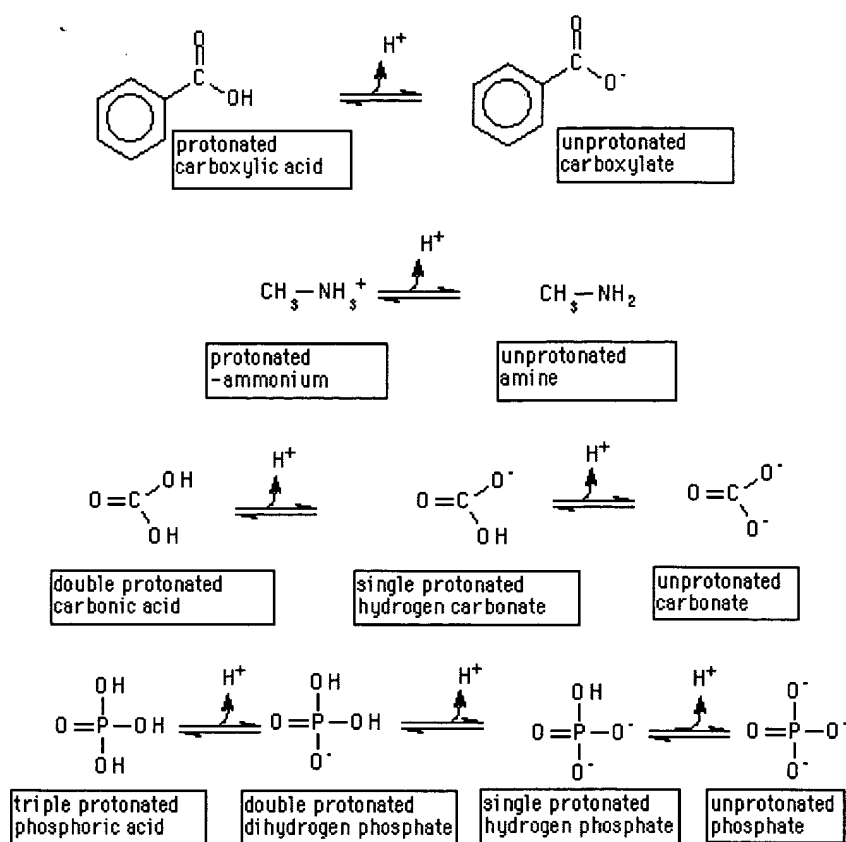
- We can often tell whether or not a substance is an acid from its name:
 - many times acids have acid in the name itself (*ascorbic acid*; *acetylsalicylic acid*)
 - also common we find the prefix hydrogen in the name (*hydrogen carbonate*)
 - drugs that are in the acid form have the term hydrochloride in it (*phenylephrine hydrochloride*)
 - the acid form of ammonium or amines can have the suffix -ium in the name (*cetyl pyridinium chloride*)

Notice the acid form of things often have a counteranion (like chloride, sulfate, tartrate)

- We can also often tell whether or not a substance is a base from its name:
 - the name of the base often ends in -ate (*acetate*; *lactate*)
 - drugs in the base form often end with sodium (*warfarin sodium*)
 - drug names can also contain the word base or amine in the name free base cocaine; (*dopamine*)

Notice the base form of substances often have a counteranion (like sodium or calcium)

- We can often tell whether or not a substance is an acid from its structure: The structural formula is the most common way that those who use chemistry represent chemical compounds. There are four functional groups that you should know that have acid forms (protonated) and base forms (unprotonated). This is not an exhaustive list, but these four are very, very common and important. You should first learn them and their properties as isolated groups (the four given forms) and then recognize them in more complicated structural formulas.



- The **carboxylate** group tends to give up its proton easily and so it takes a high hydrogen concentration (low **pH**) to protonate the group. The pKa ranges from 2-5 for the carboxylic acid group. Common substances containing the carboxylic acid group include acetic acid (found in vinegar), lactic acid (responsible for sore muscles), formic acid (the bite in stinging ants), benzoic acid, citric acid (citrus fruits), ascorbic acid (Vitamin C), and all amino acids.
- The **amino** functional group tends to hold on to its protons and so even at low hydrogen concentrations (high **pH**), the amino group is protonated. The pKa ranges from 8-10. All amino acids contain both of these functional groups; some amino acids contain additional groups that can be protonated or unprotonated.
- The **carbonate** functional group is a special but very important case of the carboxylate group. The three forms are common throughout nature. Carbonic acid decomposes to carbon dioxide and water. Carbonic acid can also have double protonated, single protonated, and unprotonated forms.
- The **phosphate** group is also common throughout nature. Phosphoric acid has three protons so there are three possible anionic forms, dihydrogen phosphate, hydrogen phosphate, and phosphate.

The amino acid, glycine, contains both the amine group (which can be protonated or unprotonated) and the carboxylic acid group (which also can be protonated or unprotonated).

Note I have added additional information, the pKa. This number tells a chemist the strength of that acid. The lower the pKa the easier it is for that form to give up a proton. The values above show that the double protonated form (carbonic acid) gives up a proton easier than the hydrogen carbonate form. Of course the unprotonated form doesn't have any protons to give so it doesn't have a pKa.

The importance of the pKa value.

The pKa tells us how easily a substance gives up a proton. It also tells you the **pH** value needed to get a proton onto the unprotonated form. It also tells us how the **pH** of the solution makes a particular structure protonated or unprotonated.

- If the **pH** is adjusted so its value is less than the pKa then the protonated form predominates
- If the **pH** is adjusted so its value is greater than the pKa then the unprotonated form predominates
- If the **pH** = pKa then there is an equal amount of protonated and unprotonated form.

You have to know how to use these statements backwards and forwards, awake and in your sleep, in this course and every course you will ever take...THIS IS IMPORTANT.

How do we use this statement in the carbonic acid case?

- Suppose I have a solution of sodium hydrogen carbonate (baking soda). If I lower the **pH** to 2 by adding an acid, then the protonated form (carbonic acid) is formed (2 is less than 4.5). What you would observe is bubbling (because carbonic acid is unstable forming CO_2 and water).
- Suppose instead I have a solution of carbonic acid and adjust its **pH** to 7.4 (which is what your blood does) then I have mostly hydrogen carbonate because at 7.4 the carbonic acid gives up one proton but not both. I would have to raise the **pH** to greater than 8.3 to get carbonate formed.
- Suppose I bubble CO_2 into water so that carbonic acid is formed, then since carbonic acid is the predominant form, the **pH** of the water must be less than 4.5.

In experiment 3, we will study the properties of buffers, solutions that contain both a weak acid and the unprotonated form of that weak acid together in solution. Examples include:

- acetic acid with sodium acetate
- sodium dihydrogen phosphate with sodium hydrogen phosphate
- benzoic acid with sodium benzoate
- anilinium chloride with aniline
- ammonium chloride with ammonia
- hydrogen carbonate with carbonate

Titration:

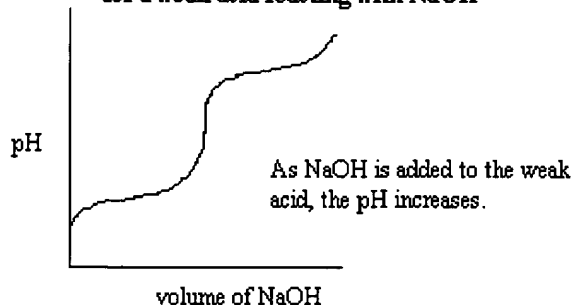
*a technique to systematically raise or lower the **pH** of a solution.*

In experiment 4 you will do a titration curve. We will restrict our titrations to adding volumes of NaOH or HCl to substances.

A titration curve is a graph of **pH** on the y axis as a function of the amount of NaOH or HCl added. If NaOH is added, the **pH** increases as NaOH is added.

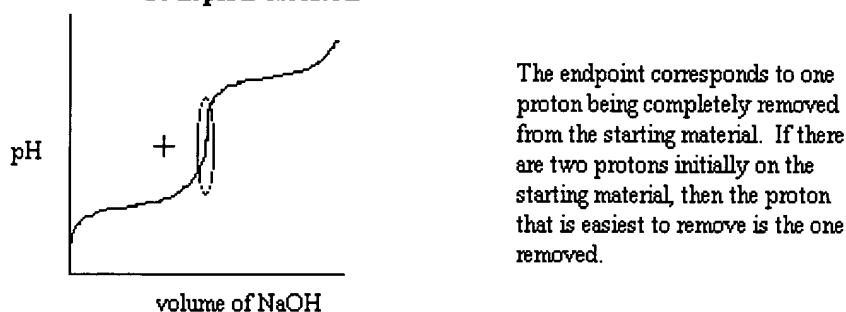
Here is a sample titration curve of a weak acid with two hydrogens/molecule:

This is a graph of a typical titration curve for a weak acid reacting with NaOH



The sharp drops are called "endpoints". Endpoints can be difficult to see. Note in the example that the first endpoint is **obvious** but you have to look hard to see the second one.

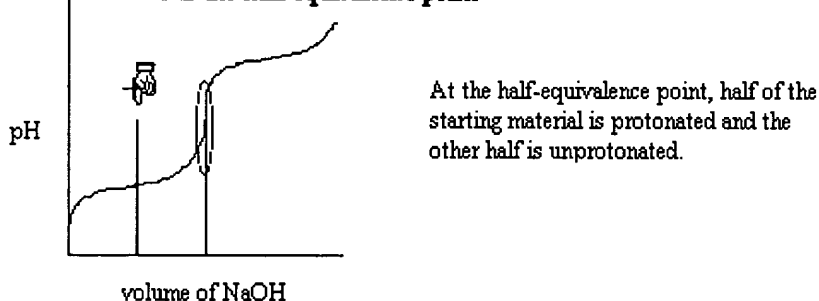
The endpoint occurs when a sharp increase in pH is observed.



The graph also shows a region where the pH drop is not sharp:

This is the buffer region. In the buffer region, the pH does change, but not as rapidly as at the endpoints. The value of the pH at the half-way point between two endpoints is the pKa.

This is the half-equivalence point



In this case it occurs at a pH of about

4. At pH values less than 4, the protonated form predominates, above pH 4, the unprotonated form predominates. The pH value is also the pKa value at this point.

Your instructor may show you how to take the derivative of the titration curve. This is easy on Graphical Analysis. At the endpoint, there is a significant change in the slope and so we get a peak on the first derivative curve. Try it>

The Experiment:

This is a team experiment (a typical team will have 4-5 students)

Your team will be given a 100 mL of a 0.1M solution of an unknown acid. The unknown might be:

- 0.1M sodium hydrogen phosphate
- 0.1M sodium dihydrogen phosphate
- 0.1M acetic acid
- 0.1M sodium hydrogen carbonate
- 0.1M sodium hydrogen sulfate
- 0.1M phosphoric acid
- 0.1M ammonium chloride
- 0.1M boric acid
- 0.2M acetic acid

You will also have 0.1M NaOH and a **pH** meter available.

Devise a way to **determine** which solution you have. You must do at least one titration and **determine** the pKa of the acid.

Here are more detailed instructions:

Experiment 1.

Measure a constant volume or constant mass of your acid. You decide a good amount. Add two drops of an indicator. We suggest phenolphthalein. Count the drops of 0.1M NaOH needed to cause the indicator to change from colorless to pink. Repeat enough times to get a value you are confident of. Compare your results with those of others in the class. **Experiment 2.**

We will have available a saturated solution of benzoic acid and sodium benzoate. We will give you one of the unknowns and ask you to identify it. We will have available 0.1M HCl and 0.1M NaOH in dropping bottles. You are to **determine** the identity of the unknown and explain your method of determining it.

Experiment 3. We will have available a four solutions.

- There will be three solutions containing 0.1M NaHPO_4 and 0.1M NaH_2PO_4 . In one solution the amount of the protonated form will be greater than the amount of the unprotonated form. In the second, the amounts of the two forms will be equal. In the third the amount of the unprotonated form will be greater than the amount of the protonated form.
- There will be a solution containing HCl and NaCl at about the same **pH** as one of the other three.

You are to identify each solution. For each solution:

1. measure the **pH**
2. pour 10 mL of mixture into a beaker and add 0.1M HCl dropwise (counting the drops) until the **pH** changes 1 **pH** unit.
3. pour 10 mL of the mixture into a beaker and add 0.1M NaOH dropwise (counting the drops) until the **pH** changes 1 **pH** unit calculate the ratio of amount of NaOH to amount of HCl.

Experiment 3b.

Pour 5 mL of the mixture you **determine** contains the dihydrogen phosphate ion into a 150mL beaker, add 100 mL of distilled water, and enough drops of bromocresol purple to see a distinct color. Then using a pipet or straw blow bubbles into the solution until the purple color no longer persists counting

the number of whole breaths needed.

Answer these questions:

- Is the purple form of bromocresol purple protonated or unprotonated?
- Why does the purple color no longer persist as you blow into the solution?

Note: it may be that your mixture is not purple, then the answer is 0 breaths.

Experiment 4

Measure 25 mL of your unknown into a small beaker. Measure the **pH** as 1M NaOH is added. Keep a table of the amount of NaOH as a function of **pH** and graph the results. Use Graphical analysis to analyze your results. Take the first derivative if your instructor requests and **determine** the endpoint, the half-endpoint and from it the pKa of your acid. Use this data to identify the unknown.

Your notebook should contain all the corroborating evidence to prove that you know what the unknown is along with data showing that you understand:

- what an endpoint is
- what a buffer is
- what an acid is
- what a base is
- how to **determine** the pKa from a titration curve
- how **pH** affects structure
- how **pH** affects color

[Download the experiment in PDF format](#)

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